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Molecular phylogenetics of deep-sea amphipods (*Eurythenes*) reveal a new undescribed species at the Porcupine Abyssal Plain, North East Atlantic Ocean

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ABSTRACT

The genus *Eurythenes* S. I. Smith in Scudder, 1882, has been the focus of integrated molecular and morphological taxonomy studies in recent years, resulting in the number of species in the genus increasing from three to eight. Samples of *Eurythenes* spp. collected using free-fall baited traps from the Porcupine Abyssal Plain (PAP), North East Atlantic Ocean, at 4850 m were examined in this study to investigate the identity of the species found using molecular barcoding methods. Mitochondrial COI analysis confirms the presence of *E. maldoror* at PAP and data from the nuclear 28S rDNA gene provides independent confirmation of this observation. A new, undescribed species that is clearly divergent from all other known species and molecular lineages was identified, which can be distinguished morphologically from known species. A specimen within the *E. magellanicus* lineage was also found at the site. We discuss the presence of these three *Eurythenes* species at the Porcupine Abyssal Plain.

1. Introduction

In recent years, the genus *Eurythenes* S. I. Smith in Scudder, 1882, has been the focus of a number of integrated molecular and morphological taxonomy studies (Havermans et al., 2013; d'Udekem d'Acoz and Havermans, 2015; Ritchie et al., 2015; Eustace et al., 2016; Havermans, 2016; Narahara-Nakano et al., 2018). Until 2004, the genus contained just two species, *Eurythenes gryllus* (Lichtenstein in Mandt, 1822), a large (up to 154 mm) well-known, and widely recorded species, found in all oceans of the world, and *Eurythenes obesus* (Chevreux, 1905), a smaller species (up to 80 mm) confined to bathypelagic depths but also known from most ocean basins (Stoddart and Lowry, 2004; Thurston and Bett, 1995). For many years, there has been considerable doubt regarding the identity of *Eurythenes gryllus,* and discussions of whether the species is truly cosmopolitan or comprised of a complex of species, can be found in a number of studies (Barnard, 1961; Bowman and Manning, 1972; France and Kocher, 1996; Ingram and Hessler, 1983; Thurston and Bett, 1995; Bucklin et al., 1987). In 2004, a third species *Eurythenes thurstoni* Stoddart and Lowry, 2004 was described, alongside a thorough redescription of the known species in the genus from type materials. Havermans et al. (2013), explored the variability of the genus on a global scale using analyses of nuclear (28S rDNA) and mitochondrial (COI and 16S rDNA) sequence data, and revealed nine lineages within the *Eurythenes* complex.

In 2015, d'Udekem d'Acoz & Havermans formally described three of the newly revealed lineages as new species and resurrected the species *Eurythenes magellanicus* (H. Milne Edwards, 1848). The addition of *Eurythenes aequilatus* Narahara-Nakano, Nakano, & Tomikawa, 2017, brought the total number of formally described species in the genus to eight: *E. gryllus* (Lichtenstein in Mandt, 1822), *E. magellanicus* (H. Milne Edwards, 1848), *E. obesus* (Chevreux, 1905), *E. thurstoni* Stoddart and Lowry, 2004, *E. andhakarae* d'Udekem d'Acoz and Havermans, 2015, *E. maldoror* d'Udekem d'Acoz and Havermans, 2015, *E. sigmiferus* d'Udekem d'Acoz and Havermans, 2015 and *E. aequilatus* Narahara-Nakano, Nakano, & Tomikawa, 2017. Additional genetically and morphologically distinct species of *Eurythenes* are awaiting description, including abyssal and hadal forms reported from the Atacama and Peru-Chile Trench (Thurston et al., 2002; Ritchie et al., 2015; Eustace et al., 2016) and the NE Atlantic (pers. obs.).

Numerous ecology and physiology studies have focussed on the

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giant deep-sea scavenger *E. gryllus sensu lato* (summarised in d'Udekem d'Acoz and Havermans, 2015). However, owing to the taxonomic uncertainty surrounding the *E. gryllus* complex, these studies will need to be revisited to confirm identity of the target species, with many likely remaining unresolved (Havermans, 2016; d'Udekem d'Acoz and Havermans, 2015). *E. gryllus sensu stricto* is a bipolar species, with a type locality in the Greenland Sea and is distributed in the Arctic and Antarctic at depths of 839–3803 m (d'Udekem d'Acoz and Havermans, 2015).

The holotype of *E. magellanicus*, described from a specimen from the stomach of a fish taken off Cape Horn, Drake Passage, in the South Atlantic, has been redescribed by Stoddart and Lowry (2004). This species has been resurrected from synonymy with *E. gryllus*. It is now known to be widely distributed, occurring in the Brazil Basin and Peru-Chile Trench region at abyssal depths (d'Udekem d'Acoz and Havermans, 2015; Ritchie et al., 2015), with recent records from the West Pacific Ocean, off Taiwan and Okinawa at bathyal depths (1300–1400 m) (Havermans, 2016, Narahara-Nakano et al., 2018).

E. obesus is characterised by the very long curved dactyls on pereopods 3–7 and a narrow, linear eye. It was originally described from a station in the North Atlantic, south of the Azores, and Stoddart and Lowry (2004) designated a neotype of the species from NE of the Cape Verde Islands. *E. obesus* is widely distributed in all oceans (128–1600 m) and is a pelagic species, collected in midwater trawls and not baited traps. There are few sequences available for the species and it is so far characterised by COI and 16S.

E. thurstoni is the smallest species of *Eurythenes, (*reaching 46 mm but attaining sexual maturity at less than 30 mm). The species was based on specimens from the Tasman Sea, off SE Australia. The species is known from localities in the western South Pacific Ocean and the western North Atlantic Ocean (for full details see Stoddart and Lowry, 2004; d'Udekem d'Acoz and Havermans, 2015). *E. thurstoni* is thought to be an epibenthic scavenger, having been taken in bottom-set baited traps, but may also be a midwater predator/scavenger like *E. obesus*, based on the relatively frequent occurrence in midwater trawls (Stoddart and Lowry, 2004). It has a shallower distribution than other species (128–1960 m) with the few abyssal records resulting from catches in midwater when the trawl was brought up (d'Udekem d'Acoz and Havermans, 2015). Genetically the species has been defined by 16S (France and Kocher, 1996) and COI (Havermans, 2016) sequences from specimens collected from widely different locations. Morphologically the species can be distinguished easily from others in the genus by the upturned ridge around the anterior margin of the head, the broadly rounded posteroventral corner of coxa 4, the long posterodistal lobe of the pereopod 7 basis, and the lack of a dorsal notch on pleonite 3.

E. andhakarae was described from the Weddell Sea in Antarctica at depths 3070–4693 m. The species has been well-defined using integrative taxonomy (28S, COI, 16S), but is very similar morphologically to *E. gryllus* and *E. magellanicus*. It can be distinguished from these two species by the comparatively narrow merus of pereopods 6 and 7, more produced anterior lobe of the head and the eye shape (d'Udekem d'Acoz and Havermans, 2015).

E. maldoror, was collected first in the NW Argentine Basin at 4602 m, but the species has been shown to be widely distributed at depths below 3000 m. Specimens have been collected in the Weddell Sea and north Argentinian Basin (d'Udekem d'Acoz and Havermans, 2015), and genetic studies suggest a wide distribution in the North Atlantic and North Pacific down to 5117 m (Havermans et al., 2013; Havermans, 2016).

E. sigmiferus is a very characteristic species with prominent dorsal carinations. It was described originally from a single specimen from the South West Atlantic, in the Brazil Basin at 4480 m. Unfortunately, only 16S sequence data are available, meaning comparisons with newly collected specimens are more difficult. There has been some confusion in the literature concerning the identity of this species, which is surprising since it has such a striking morphology. Eustace et al. (2016) provide a photograph of a specimen of what appears to be *E. sigmiferus*, but is labelled as *E. magellanicus.* The identification was apparently determined using molecular data, but it is not clear if the specimen in the photograph is the same one that was used for the molecular taxonomic identification.

E. aequilatus, the most recently described species in the genus, has been well defined using an integrated morphological and molecular approach (with 28S, H3, COI, & 16S sequences available for both the holotype and paratype). The species was described from the southern Sea of Okhotsk at depths of 1574–1582 m, and is thus far only known from the type locality (Narahara-Nakano et al., 2018).

Samples of *Eurythenes* spp. collected from the Porcupine Abyssal Plain (PAP), North East Atlantic Ocean, at 4850 m were examined in this study. The PAP has been the focus of studies of scavenging amphipods including *Eurythenes* since 1978 (Thurston, 1990) and amphipod trapping has been carried out regularly since then, producing the longest known time-series study of scavenging amphipods at an abyssal site (see Horton et al., this issue). Until the study of Havermans et al. (2013) specimens of *Eurythenes* collected at the PAP were identified as the cosmopolitan species *Eurythenes gryllus*, although morphological variations among these specimens and others had been reported before (Thurston and Bett, 1995; Thurston et al., 2002). The new studies cast further doubt on the specific identity of the specimens of *Eurythenes* in our collections and therefore this research sets out to clarify the identity of the species found at the PAP, using an integrated morphological and molecular approach. *Eurythenes obesus* is also known from the North Atlantic generally, and PAP specifically, but since it is a morphologically distinct species it is not considered problematic and will not be considered further here.

2. Methods

Amphipod samples were collected using free-fall baited traps deployed during two research cruises to the Porcupine Abyssal Plain on R.R.S. *James Cook* and R.R.S. *Discovery* in 2014 and 2017. The trap-rig consisted of a frame, traps, buoyancy, an acoustic release and a ballast weight located in a recess on the underside of the frame. There are four traps on the rig; two benthic and two epibenthic traps (1 m above bottom) set at 90°to each other. Each trap was 500 mm long by 290 mm internal diameter with a double funnel entrance (45 mm then 35 mm apertures) at one end, baited with a single mackerel (*Scomber scombrus*). On retrieval of the trap, contents were fixed in 100% ethanol and stored at −20 °C. Ethanol was replaced during the sorting process, immediately after samples were returned to the laboratory, and samples were kept at −20 °C until DNA subsamples had been taken.

Amphipod traps were set at two stations each year (Table 1). Individuals of *Eurythenes* spp. were selected from the samples and placed into individual specimen tubes for further identification and tissue subsampling. Each specimen was given an individual code concatenating the station number, morphotype and specimen number (e.g. DY077/083_A26). Successfully sequenced individuals are detailed in Table 1.

Prior to molecular analyses *Eurythenes* specimens were separated morphologically into two different entities, *Eurythenes* sp. DISCOLL_PAP_A (Fig. 1) and *Eurythenes* sp. DISCOLL_PAP_B (Fig. 2). Identification to species level was attempted using the identification keys in Stoddart and Lowry (2004), d'Udekem d'Acoz and Havermans (2015) and Narahara-Nakano et al. (2018). However, since the specimens were in most cases immature or juvenile (less than 25 mm) and there was the possibility of new species being present, the keys could not be relied upon to separate species (as indicated in d'Udekem d'Acoz and Havermans (2015: 9)). The two morphotypes were present in all samples studied, but *Eurythenes* sp. DISCOLL_PAP_A was present in higher numbers and was provisionally identified as belonging to the species *Eurythenes maldoror* while *Eurythenes* sp. DISCOLL_PAP_B could not be ascribed to any of the known species and was believed to belong

Table 1
Station data for specimens collected at the Porcupine Abyssal Plain and used for sequencing in this study.

Specimen No.	Station No.	Deployment Date	Decimal Latitude	Decimal Longitude	Depth (M)	INSDC Accession Numbers	
						COI	28S
JC085/19_A4	JC085#19	22/04/14	48 59.367	16 30.400	4843	MN832607	MN847704
JC085/19_A5	JC085#19	22/04/14	48 59.367	16 30.400	4843	MN832608	MN847705
JC085/19_A6	JC085#19	22/04/14	48 59.367	16 30.400	4843	–	MN847706
JC085/19_A7	JC085#19	22/04/14	48 59.367	16 30.400	4843	–	MN847707
JC085/19_A8	JC085#19	22/04/14	48 59.367	16 30.400	4843	–	–
JC085/19_A9	JC085#19	22/04/14	48 59.367	16 30.400	4843	–	MN847708
JC085/19_A10	JC085#19	22/04/14	48 59.367	16 30.400	4843	–	MN847709
JC085/19_B3	JC085#19	22/04/14	48 59.367	16 30.400	4843	MN832609	–
JC085/19_B8	JC085#19	22/04/14	48 59.367	16 30.400	4843	MN832610	MN847710
DY077/61_A20	DY077#61	23/04/17	49 00.423	016 23.820	4846	MN832598	MN847696
DY077/61_A23	DY077#61	23/04/17	49 00.423	016 23.820	4846	MN832599	MN847697
DY077/61_A24	DY077#61	23/04/17	49 00.423	016 23.820	4846	MN832600	–
DY077/61_A26	DY077#61	23/04/17	49 00.423	016 23.820	4846	MN832601	MN847698
DY077/83_A7	DY077#83	25/04/17	49 00.442	016 25.168	4846	MN832597	MN847695
DY077/83_B4	DY077#83	25/04/17	49 00.442	016 25.168	4846	MN832603	MN847700
DY077/83_B2	DY077#83	25/04/17	49.00.442	016 25.168	4846	MN832602	MN847699
DY077/83_B6	DY077#83	25/04/17	49 00.442	016 25.168	4846	MN832604	MN847701
DY077/83_B10	DY077#83	25/04/17	49 00.442	016 25.168	4846	MN832605	MN847702
DY077/61_B13	DY077#83	25/04/17	49 00.442	016 25.168	4846	MN832606	MN847703

Fig. 1. Photograph of *Eurythenes* sp. DISCOLL_PAP_A (specimen DY077/61_A20) = *Eurythenes maldoror.*

Fig. 2. Photograph of *Eurythenes* sp. DISCOLL_PAP_B (specimen DY077/83_B4) = *Eurythenes* sp. nov.

Fig. 3. Photograph of *Eurythenes* sp. specimen DY07783_B2. = *Eurythenes* cf. *magellanicus.*

to a species new to science. Specimens are stored in the Discovery Collections at the National Oceanography Centre (DISCOLL, NOC, UK; https://www.gbif.org/grscicoll/institution/74ae2bc3-e5a8-443f-bc8b-89cc223500d1).

From each individual morphotype identified, two pleopods and some muscle tissue were dissected and placed in 100% ethanol for molecular analysis. DNA extraction of tissue samples was conducted using the Qiagen DNeasy® Blood and Tissue extraction kit following the manufacturer's protocol. COI was amplified using a new forward primer, AmpCOIF2 (5′-GRTCTGARCTCAGYRGRCCRGG-3′) and a universal reverse primer HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) (Folmer et al., 1994). Universal 28S primers (Hou et al. 2007, Raupach et al., 2010), 28F and 28R, were used to amplify 28S as well as newly developed forward (28S_seq_FWD: 5′-TGGAGCTTGGAAGGCTTCC-3′) and reverse (28S_seq_REV 5′-TGGACTTAACACCACGCGAGT-3′) primers. PCR products were cleaned using a MultiScreen-PCR96 Filter Plates (Millipore), and purified PCR products were bidirectionally sequenced.

Previously published COI and 28S sequences were retrieved from Genbank (see Table 2 for accession numbers and references). The ClustalW algorithm in MEGA X was used to align and edit COI sequences (Thompson et al., 1994, Kumar et al., 2018). MAFFT was used to align 28S sequences. MrBayes was used to estimate phylogenies and node support (Ronquist and Huelsenbeck, 2003). The general time reversable model with gamma-distributed rate variation and a proportion of invariant sites was applied for both genes. A total of 1,100,000 steps in the Markov Chain were sampled thinning every 1000 steps. Heating was applied to each run, employing 20 chains with a temperature of 0.1, and 5 independent analyses were conducted from random starting trees to ensure convergence. The first 10% of posterior samples from each independent run were discarded, and the five runs were combined for final posterior inference. Analyses were conducted for each gene separately and on the combined dataset. For the combined data analysis, all parameters were unlinked between partitions except topology and branch lengths.

Table 2
Sequences of *E. aequilatus* (AEQ) *E. andhakarae* (AND), *E. gryllus* (GRL), *E. magellanicus* (MAG), *Eurythenes maldoror* (MAL), *E. obesus* (OBE), and the undescribed lineages *Eurythenes* sp. Hadal (HAD), *Eurythenes* sp. Abyssal (ABY) (From Ritchie et al., 2015), and UNK 1 and UNK 2 representing two unknown taxa; obtained for this study from GenBank (from Havermans, 2016). Holotypes are represented in **BOLD**, n.d. – no data available.

Species Code	Specimen Code	Sample Location	INSDC Accession Numbers		Reference
			COI	28S	
UNK1	*WDL-d1*	Weddell Sea	KX078273	n. d.	Havermans (2016)
UNK2	*MOZ-1*	Mozambique Channel	KX078271	n. d.	Havermans (2016)
ABY	*E. sp. 2A-2*	Peru-Chile Trench, Abyssal	KP713958	n. d.	Ritchie et al. (2015)
ABY	*E. sp. 2A-1*	Peru-Chile Trench, Abyssal	KP713957	n. d.	Ritchie et al. (2015)
HAD	*E. sp. 1A-1*	Peru-Chile Trench, Hadal	KP713955	n. d.	Ritchie et al. (2015)
HAD	*E. sp. 1A-2*	Peru-Chile Trench, Hadal	KP713956	n. d.	Ritchie et al. (2015)
AEQ	*OKI-1*	Hokkaido- Sea of Okhotsk	LC229095	LC229093	Narahara-Nakano et al. (2018)
AEQ	***OKI-2***	**Hokkaido- Sea of Okhotsk**	**LC229094**	**LC229092**	Narahara-Nakano et al. (2018)
AND	***Ant-a1***	**East of Antarctic Peninsula**	**JX887112**	**JX887078**	Havermans et al. (2013)
AND	*Ant-a2*	East of Antarctic Peninsula	JX887116	JX887077	Havermans et al. (2013)
AND	*WDI-a1*	Eastern Weddell Sea	JX887114	JX887075	Havermans et al. (2013)
AND	*WDL-a2*	Eastern Weddell Sea	JX887138	n. d.	Havermans et al. (2013)
AND	*WDL-a4*	Weddell Sea	KX078270	n. d.	Havermans (2016)
AND	*WDL-a5*	Weddell Sea	KX078269	n. d.	Havermans (2016)
AND	*WDL-a6*	Weddell Sea	KX078268	n. d.	Havermans (2016)
AND	*WDL-a7*	Weddell Sea	KX078267	n. d.	Havermans (2016)
AND	*WDL-a12*	Weddell Sea	KX078257	n. d.	Havermans (2016)
AND	*WDL-b2*	Weddell Sea	JX887119	n. d.	Havermans et al. (2013)
AND	*WDL-b3*	Weddell Sea	n. d.	JX887081	Havermans et al. (2013)
AND	*WDL-b4*	Weddell Sea	JX887115	JX887078	Havermans et al. (2013)
AND	*WDL-b6*	Weddell Sea	n. d.	JX887082	Havermans et al. (2013)
AND	*WDL-c1*	Weddell Sea	JX887117	JX887079	Havermans et al. (2013)
AND	*WDL-c2*	Weddell Sea	JX887113	n. d.	Havermans et al. (2013)
AND	*WDL-c3*	Weddell Sea	n. d.	JX887080	Havermans et al. (2013)
AND	*WDL-c4*	Weddell Sea	JX887118	n. d.	Havermans et al. (2013)
AND	*SS-1*	South Sandwich Island	JX887120	JX887076	Havermans et al. (2013)
GRL	*Ant-b1*	North of Antarctic Peninsula	JX887140	JX887088	Havermans et al. (2013)
GRL	*Ant-b2*	North of Antarctic Peninsula	JX887139	JX887089	Havermans et al. (2013)
GRL	*Arctic-a1*	Eastern Fram Strait	JX887129	n. d.	Havermans et al. (2013)
GRL	*Arctic-a3*	Eastern Fram Strait	JX887127	JX887085	Havermans et al. (2013)
GRL	*Arctic-a4*	Eastern Fram Strait	JX887128	JX887086	Havermans et al. (2013)
GRL	*Arctic-a5*	Eastern Fram Strait	JX887130	JX887087	Havermans et al. (2013)
GRL	*Arctic-a6*	Eastern Fram Strait	JX887126	n. d.	Havermans et al. (2013)
GRL	*Arctic-b1*	Eastern Fram Strait	JX887131	JX887084	Havermans et al. (2013)
GRL	*Arctic-b2*	Eastern Fram Strait	JX887132	JX887083	Havermans et al. (2013)
GRL	*Arctic-c1*	Svalbard Archipelago	JX887148	JX887095	Havermans et al. (2013)
GRL	*Arctic-c4*	Svalbard Archipelago	JX887147.1	JX887109	Havermans et al. (2013)
GRL	*Arctic-c5*	Svalbard Archipelago	JX887149	n. d.	Havermans et al. (2013)
GRL	***Arctic-c6***	**Svalbard Archipelago**	**JX887147**	**JX887109.1**	Havermans et al. (2013)
GRL	*Arctic-c7*	Svalbard Archipelago	JX887150	JX887096	Havermans et al. (2013)
GRL	*Arctic-c8*	Svalbard Archipelago	KX078265	n. d.	Havermans et al. (2013)
GRL	*Arctic-c9*	Svalbard Archipelago	KX078264	n. d.	Havermans et al. (2013)
GRL	*Arctic-c10*	Svalbard Archipelago	KX078263	n. d.	Havermans et al. (2013)
GRL	*Arctic-c11*	Svalbard Archipelago	KX078262	n. d.	Havermans et al. (2013)
GRL	*Arctic-c12*	Svalbard Archipelago	KX078261	n. d.	Havermans et al. (2013)
GRL	*KGI-a1*	King George Island	JX887134	n. d.	Havermans et al. (2013)
GRL	*KGI-b1*	King George Island	JX887135	n. d.	Havermans et al. (2013)
GRL	*KGI-c1*	King George Island	JX887142	JX887090	Havermans et al. (2013)
GRL	*KGI-c2*	King George Island	JX887141	JX887091	Havermans et al. (2013)
GRL	*KGI-c3*	King George Island	JX887133	n. d.	Havermans et al. (2013)
GRL	*KGI-c4*	King George Island	JX887136	JX887092	Havermans et al. (2013)
GRL	*KGI-c5*	King George Island	n. d.	JX887093	Havermans et al. (2013)
GRL	*KGI-c6*	King George Island	n. d.	JX887094	Havermans et al. (2013)
GRL	*KERG-a1*	Kerguelen Island	KX078254	n. d.	Havermans (2016)
GRL	*KERG-a2*	Kerguelen Island	KX078253	n. d.	Havermans (2016)
GRL	*KERG-a3*	Kerguelen Island	KX078252	n. d.	Havermans (2016)
GRL	*KERG-a4*	Kerguelen Island	KX078251	n. d.	Havermans (2016)
GRL	*KERG-a5*	Kerguelen Island	KX078250	n. d.	Havermans (2016)
GRL	*KERG-b1*	Kerguelen Island	KX078249	n. d.	Havermans (2016)
MAG	*BraB-1*	Brazil Basin	n. d.	JX887101	Havermans et al. (2013)
MAG	*BraB-2*	Brazil Basin	JX887144	JX887102	Havermans et al. (2013)
MAG	*BraB-3*	Brazil Basin	JX887143	JX887100	Havermans et al. (2013)
MAG	***BraB-4***	**Brazil Basin**	**JX887145.1**	**JX887097**	Havermans et al. (2013)
MAG	*BraB-5*	Brazil Basin	JX887145	JX887099	Havermans et al. (2013)
MAG	*BraB-6*	Brazil Basin	n. d.	JX887098	Havermans et al. (2013)
MAG	*BraB-7*	Brazil Basin	JX887146	n. d.	Havermans et al. (2013)
MAG	*OKI-3*	Hokkaido- Sea of Okhotsk	LC192881	LC192880	Narahara-Nakano et al. (2018)
MAG	*TAI-1*	Off Taiwan	KX078274	n. d.	Havermans (2016)
MAL	*ArgB-1*	Argentine Basin	JX887121	JX887105	Havermans et al. (2013)
MAL	*ArgB-2*	Argentine Basin	JX887137	JX887106	Havermans et al. (2013)

(*continued on next page*)

Table 2 (*continued*)

Species Code	Specimen Code	Sample Location	INSDC Accession Numbers		Reference
			COI	28S	
MAL	*ArgB-3*	Argentine Basin	JX887125	n. d.	Havermans et al. (2013)
MAL	*ArgB-4*	Argentine Basin	JX887124	JX887108	Havermans et al. (2013)
MAL	*ArgB-5*	Argentine Basin	JX887122	JX887107	Havermans et al. (2013)
MAL	***ArgB-7***	**Argentine Basin**	**JX887152**	**n. d.**	Havermans et al. (2013)
MAL	*ArgB-8*	Argentine Basin	n. d.	JX887111	Havermans et al. (2013)
MAL	*ArgB-9*	Argentine Basin	JX887151	JX887110	Havermans et al. (2013)
MAL	*Ant-a3*	East of Antarctic Peninsula	JX887123	JX887103	Havermans et al. (2013)
MAL	*Ant-a4*	East of Antarctic Peninsula	GU109270	JX887104	Havermans et al. (2013)
MAL	*WDL-a8*	Weddell Sea	KX078266	n. d.	Havermans (2016)
MAL	*WDL-a9*	Weddell Sea	KX078260	n. d.	Havermans (2016)
MAL	*WDL-a10*	Weddell Sea	KX078259	n. d.	Havermans (2016)
MAL	*WDL-a11*	Weddell Sea	KX078258	n. d.	Havermans (2016)
MAL	*WDL-a13*	Weddell Sea	KX078256	n. d.	Havermans (2016)
MAL	*WDL-a14*	Weddell Sea	KX078255	n. d.	Havermans (2016)
MAL	*WDL-a15*	Weddell Sea	KX078240	n. d.	Havermans (2016)
OBE	*E. sp. 9151*	Peru-Chile Trench, Bathyal	KP713954	n. d.	Ritchie et al. (2015)
OBE	***Eob-C103***	**Southern Atlantic Ocean**	**Eob-C103**	**n. d.**	d'Udekem d'Acoz and Havermans (2015)
OUTGROUP	*Bathycallisoma schellenbergi*	Kermadec Trench	KP713937	n. d.	Ritchie et al. (2015)
OUTGROUP	*Abyssorchomene nodimanus*	Weddell Sea	GU109260.1	GU109205.1	Havermans et al., 2010

3. Results

The mitochondrial COI analysis (Fig. 4), including our specimens as well as those from other studies (see Table 2 for accession numbers and references), confirms the presence of *E. maldoror* at PAP (designated *Eurythenes* sp. DISCOLL_PAP_A), which was collected during both cruises JC085 and DY077. Data from the nuclear 28S rDNA gene (Fig. 5) provides independent confirmation of this observation.

A specimen within the *E. magellanicus* lineage was also found at PAP. The phylogenetic placement of this specimen was confirmed independently by both mitochondrial COI and nuclear 28S. However, the individual (*Eurythenes* sp. DY077/83_B2) was more closely related to specimens identified as *E. magellanicus* collected from the western Pacific (OKI-3 (Narahara-Nakano et al., 2018) and TAI-1 (Havermans, 2016)) than to the Atlantic lineages from the Brazil Basin (BraB-4, BraB-5 and BraB-7 (Havermans et al., 2013)), which include the *E. magellanicus* voucher sequence. This provides further evidence supporting the possibility that *E. magellanicus* is composed of several species (Havermans et al., 2013). However, geographical sampling and genetic data for *E. magellanicus* is sparse and therefore with the data that currently exists, it is not possible to resolve the taxonomic composition or geographical distributions of the *E. magellanicus* complex. Therefore, we refer to this specimen as *Eurythenes* cf. *magellanicus*.

We identify a third species present in the samples from PAP that is clearly divergent from all other known species and molecular lineages. All individuals from this new species are of morphotype *Eurythenes* sp. DISCOLL_PAP_B. Specimens from this new taxon were found in samples collected during both cruises DY077 and JC085. The COI mitochondrial locus and the 28S nuclear locus both support this conclusion (Figs. 4-6).

4. Discussion

We confirm the presence of four *Eurythenes* species at PAP: *Eurythenes obesus*, *E. maldoror*, a member of the *E. magellanicus* complex, and a new lineage *Eurythenes* sp. DISCOLL_PAP_B. These inferences were confirmed using both mitochondrial and nuclear gene sequences. For the purpose of this study, the combined nuclear 28S and mitochondrial COI data provide sufficient resolution to identify samples collected at PAP to the species level. Relatively recently diverged lineages are well-resolved in the mitochondrial COI (Fig. 4), nuclear 28S (Fig. 5), and combined (Fig. 6) phylogenies, however the phylogenetic relationships of the deeper nodes are not well resolved with the current data. Significant variability in taxonomic coverage exists between the mitochondrial COI and the nuclear 28S databases, requiring the use of more divergent outgroups in the 28S and combined analyses as well as fewer taxa in the ingroup. The selection of outgroup, in this instance, significantly affects the inferred topologies, creating potential phylogenetic artefacts that are worth noting. For example, the root placement occurs at the base of *E. gryllus* for both the 28S and combined analyses, but the addition of *E.* sp. MOZ −1 and *E.* sp. WDL d1 as well as *B. schellenbergi* suggest an alternative root placement in the COI analysis. The COI phylogeny produced by removing these taxa produces a similar root placement to the 28S and combined analyses. It is possible that the root placement in the 28S and combined analyses is an artefact due to saturation at these levels of divergence. Inclusion of the less divergent taxa in the COI analysis appears to stabilize the root position to a degree, though the placement of deeply branching lineages are still not well resolved. Therefore, we suggest caution in interpreting deep phylogenetic relationships implied by the current study.

A preliminary study of the two abundant entities found at PAP showed that they could be separated morphologically and thus should be considered as pseudo-cryptic rather than cryptic species. The classification as pseudo-cryptic (i.e. morphologically diagnosable) species is discussed in Fišer et al. (2018). Morphological discrimination is based largely on characteristics of pereopod 7 (basis widely expanded with a square, flattened posterodistal lobe in PAP_A (*E. maldoror*) versus a narrowly expanded with a distinctly angled posterodistal lobe in PAP_B (the new species), but also minor characters of epimeron 2 (tooth on posterodistal corner elongate and acute in PAP_A versus shorter, less acute in PAP_B), pereon and pleosome carination (slight carination of the latter pereon and pleosome segments with a sigmoidal pleosomite 3 and urosomite 1 in PAP_A, versus pereonites less carinate and less distinct sigmoidal shape on pleosomite 3 and urosomite 1 in PAP_B), and gnathopod 1 and 2 shape (carpus of G1 shorter and G2 distally widened in PAP_B compared to PAP_A). Smaller specimens of the new species (PAP_B) were also observed to have a reddish tint to the extremities of all appendages and edges of the pereonites and pleonites, although this may be diet-related. These morphological differences are very slight and require close examination of specimens. A thorough characterisation of all morphological characters will be needed before the new species (PAP_B) can be separated confidently from known *Eurythenes* species.

As noted by d'Udekem d'Acoz and Havermans (2015) the description of any new species of *Eurythenes* should be based on mature individuals owing to the ontogenetic changes apparent in all better-known species. Descriptions should be based on individuals larger than

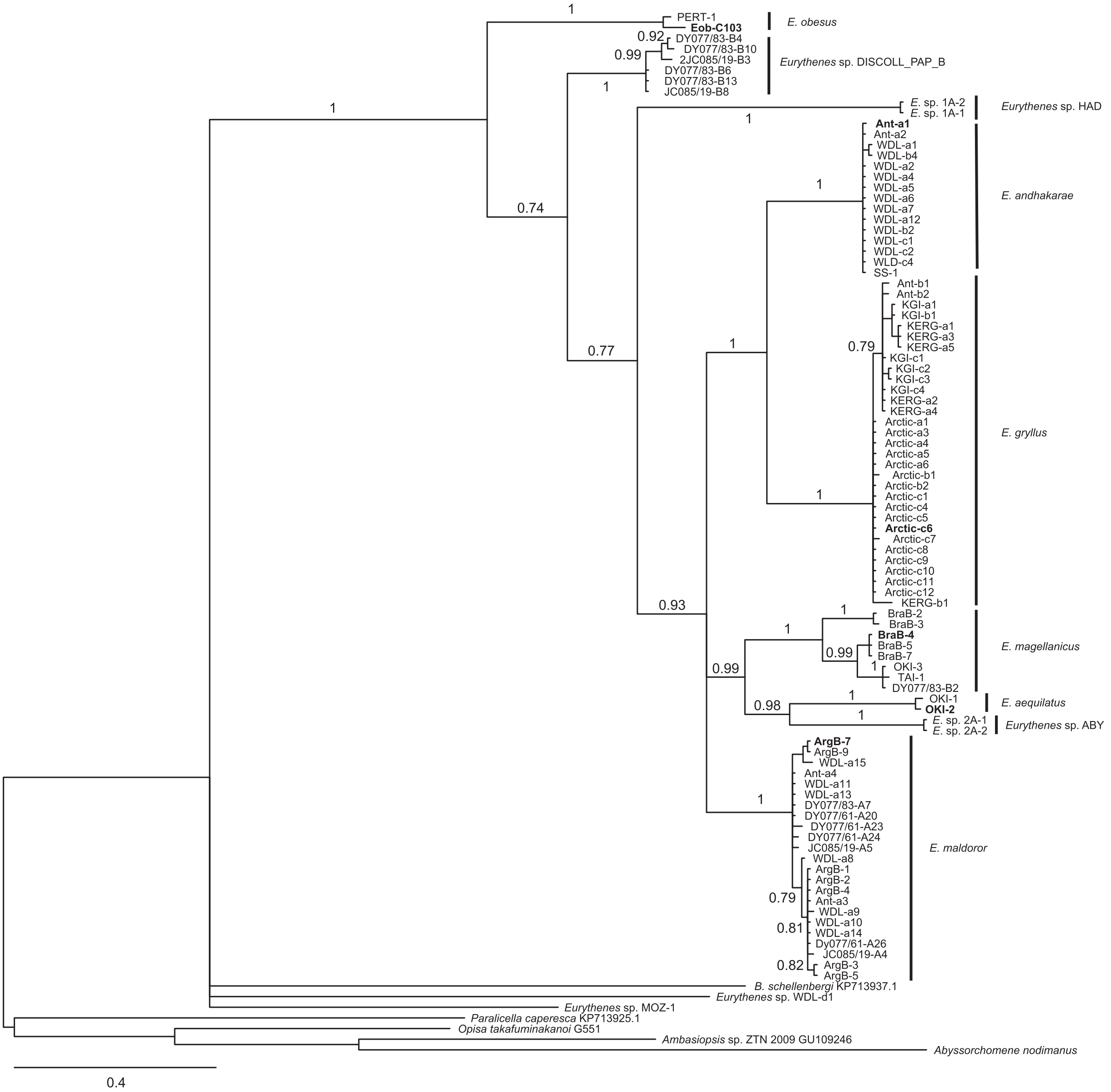

Fig. 4. Bayesian phylogeny based on mitochondrial COI data. Numbers on nodes are posterior probabilities. The COI data strongly supports the existence of the new species, *Eurythenes* sp. DISCOLL_PAP_B, and the presence of both *E. magellanicus* and *E. maldoror*. The phylogenetic relationship of *Eurythenes* sp. DISCOLL_PAP_B is not well resolved with the COI data.

25 mm and accompanied by molecular vouchers, as the current issues of identification and characterisation of species within the *Eurythenes gryllus* complex can only be resolved by detailed and careful analyses of a series of individuals over a range of sizes. The numerous specimens from the Porcupine Abyssal Plain time series (Horton et al., this issue) collected prior to cruises JC085 and DY077 were fixed in 4% formaldehyde prior to transfer to 80% ethanol, and so are unsuitable for molecular characterisation. This makes the description of the new species more problematic. Although we have defined the new species here using molecular methods, the specimens used were all small. There are 5754 specimens of *Eurythenes* available from the PAP time series that now need to be sorted and reidentified in order to find larger individuals of the new species that can be identified morphologically. Only when this work is completed, can a description of the new species based on mature individuals and a wide size range of specimens can be prepared.

There is an additional, as yet undescribed, Atlantic *Eurythenes* species in the literature, denoted as clade Eg8 in Havermans et al. (2013), which originates from sequences from the Iceland Basin at 2900 m and the Tongue of the Ocean, Bahamas at 1309 m (France and Kocher, 1996). At present, this species is defined only on the basis of 16S and therefore cannot be compared with our specimens. Sequencing of the 16S from the PAP specimens will be needed in order determine whether clade Eg8 corresponds with our new species. Additionally, if voucher material exists of the Eg8 specimens, a morphological assessment and an investigation as to whether 28S and COI could be sequenced from them would allow comparison with our data. Evidence for bathymetric segregation of *Eurythenes* species is strong (Bucklin et al., 1987) with species confined to depths less than or greater than 3000 m (Havermans et al., 2013) with only *E. magellanicus*, as currently understood, transgressing this boundary (Havermans, 2016). The undescribed species presented as clade Eg8 (Havermans et al., 2013) is of shallow occurrence (1307–2900 m) whereas the specimen from PAP in the *E. magellanicus* lineage was trapped at 4846 m making it probable, in the absence of direct genetic evidence, that the two entities are distinct.

In addition to the finding of two well-characterised species

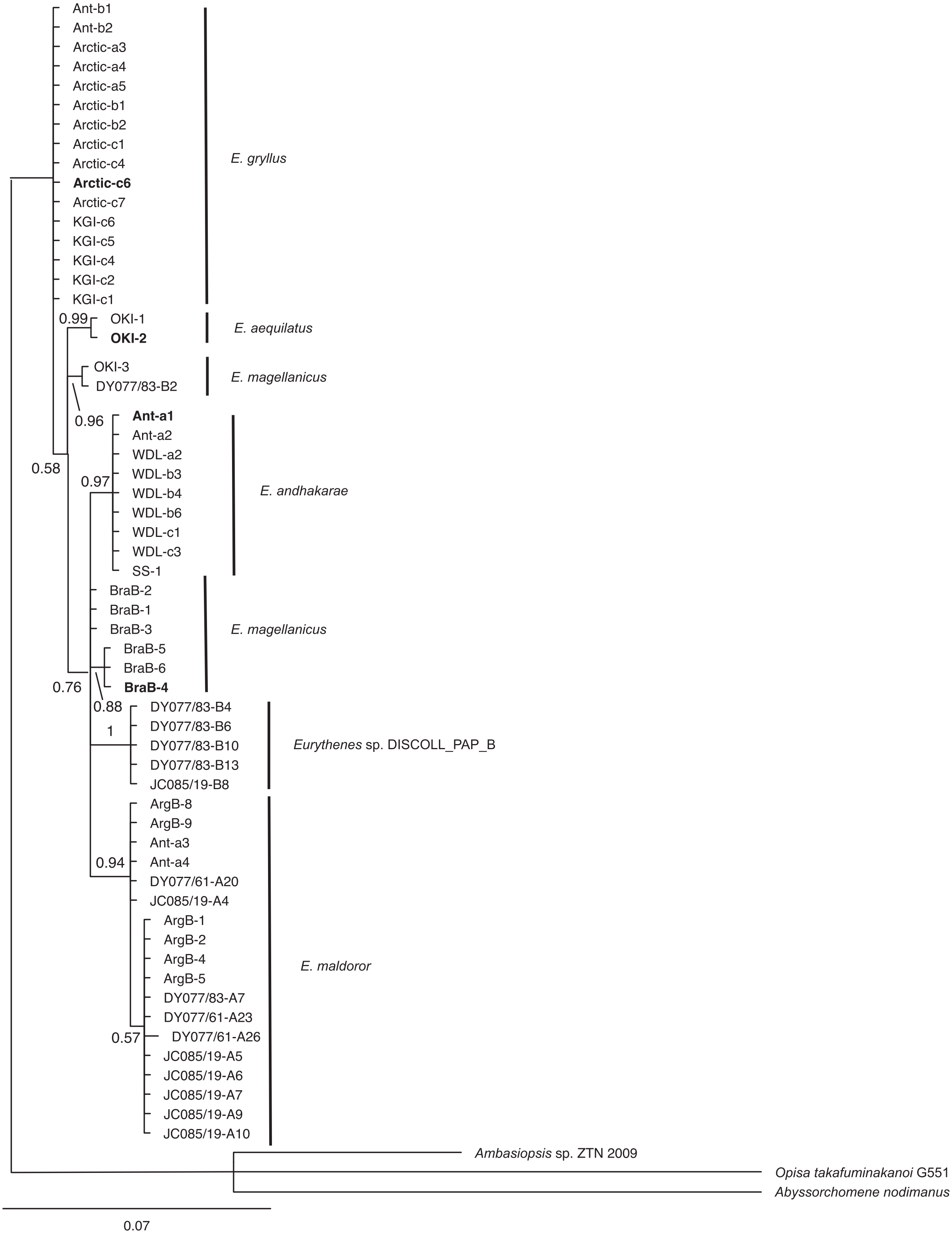

Fig. 5. Bayesian phylogeny based on nuclear 28S data. Numbers on nodes are posterior probabilities. The 28S data strongly supports the existence of the new species, *Eurythenes* sp. DISCOLL_PAP_B, and the presence of both *E. magellanicus* and *E. maldoror*. However, phylogenetic relationships among species including *Eurythenes* sp. DISCOLL_PAP_B are not well resolved with the 28S data alone.

(*Eurythenes maldoror* and the new species *Eurythenes* sp. DISCOLL_PAP_B), there was a single specimen from the *Eurythenes magellanicus* lineage (specimen *Eurythenes* sp. DY077/83_B2; Fig. 3). This specimen was not recognised during the preliminary sorting as differing from PAP_A and PAP_B, hence the allocation of the coding. Our results place this specimen closer to specimens collected from the western Pacific (OKI-3 (Narahara-Nakano et al., 2018) and TAI-1 (Havermans, 2016)) than to the Atlantic lineages from the Brazil Basin (BraB-4, BraB-5 and BraB-7 (Havermans et al., 2013)), which include the *E. magellanicus* voucher sequence. It is likely that *E. magellanicus*

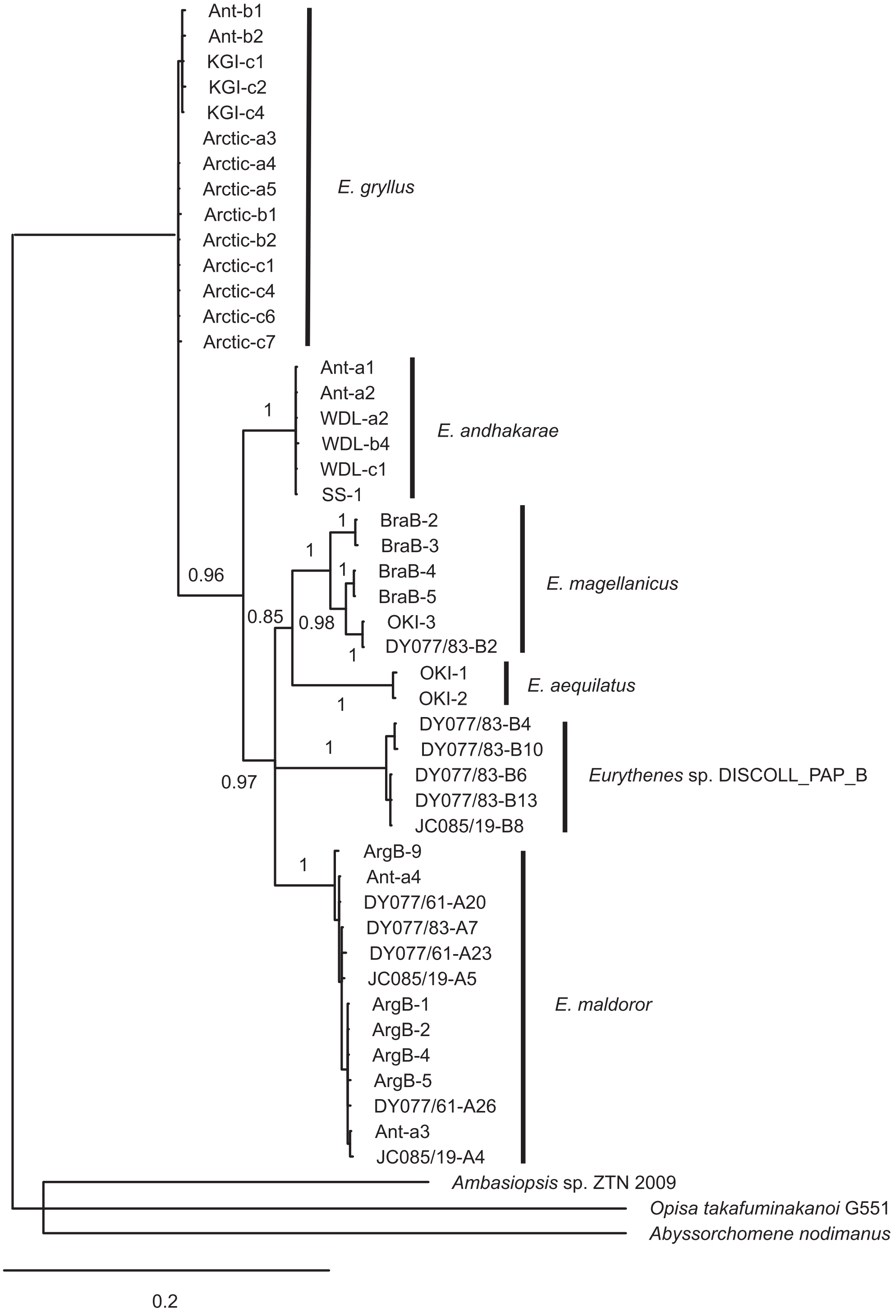

Fig. 6. Combined phylogeny using both the COI and 28S sequence data. Numbers on nodes are posterior probabilities. The multilocus data strongly supports the existence of the new species, *Eurythenes* sp. DISCOLL_PAP_B, and the presence of both *E. magellanicus and E. maldoror.*

represents a complex of several species and further work on characterising this lineage is needed. The finding of *E. magellanicus* in the North Atlantic adds to our knowledge of this species' known wide distribution in the South Atlantic, South and West Pacific Oceans at both bathyal and abyssal depths (Havermans, 2016; Narahara-Nakano et al., 2018). The fact that our North Atlantic specimens are more closely related to the Pacific specimens than the South Atlantic specimens is interesting, and potential hypotheses to explain dispersal have been discussed in Havermans (2016). However, we agree with the conclusions of Havermans (2016) that it is unlikely that the strongly swimming *Eurythenes* species are greatly influenced by topographic and hydrographic barriers to dispersal, and that a full understanding of the distribution of these taxa must await further samples and data.

Further work is also needed to clarify the species *Eurythenes sigmiferus.* It would be most useful to attempt to sequence COI, 28S genes from the holotype specimen in addition to 16S as there appear to be some conflicts in use of the 16S sequences alone for molecular taxonomic identification (Eustace et al., 2016; Havermans, 2016). It should also be mentioned here that the species denoted as *Eurythenes* sp. 3 in Narahara-Nakano et al. (2018) characterised by 16S sequences from specimens from the Gulf of Mexico (and originally published in Escobar et al., 2010) and from the Brazil Basin (Clade Eg6 in Havermans et al., 2013) are the only sequences known for *E. sigmiferus*. We have specimens in our collections collected in the Gulf of Mexico (from Escobar et al., 2010) and can confirm that morphologically these are *E. sigmiferus*. In addition, we have in our collections the specimen mentioned in d'Udekem d'Acoz and Havermans (2015) as appearing in a photograph from the ECOMAR expeditions, and can confirm that it appears to be conspecific with *E. sigmiferus*. Unfortunately attempts to sequence this specimen were unsuccessful. Clarification of the identity of known species of the giant amphipod genus *Eurythenes* is particularly important as we become more aware of the challenges in their identification. It is clear that species of this genus are morphologically very similar (having been identified until recently as *Eurythenes gryllus* sensu lato) and now increasingly, molecular data (including COI, 28S and 16S) are being relied upon to provide species identifications. There are known issues with the identification of juveniles owing to ontogenetic variability, and there are certainly more species in the genus awaiting characterisation. Therefore ensuring the correct identity of the known species via robust molecular and morphological methods is critical to strengthen our understanding. Sequencing additional independent genes (i.e. nuclear) to increase genetic coverage would be useful to resolve these issues and to aid future studies applying molecular identification methods to the genus.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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